

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/68, C07H 21/00, C07F 9/24</b>		<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 98/20166</b>
			<b>(43) International Publication Date:</b> 14 May 1998 (14.05.98)
<b>(21) International Application Number:</b> PCT/US97/20444			<b>[AT/US];</b> 3899 Haines Street #8-308, San Diego, CA 92109 (US). JURINKE, Christian [DE/DE]; Grope Hall 68, D-22115 Hamburg (DE). VAN DEN BOOM, Dirk [DE/DE]; Forsthausstrasse 8, D-63303 Preiech (DE). XIANG, Guobing [CN/US]; Apartment: 23, 11381 Zapata Avenue, San Diego, CA 92126 (US). LOUGH, David, M. [GB/GB]; 32 Deanhead Road, Eyemouth, Berwickshire TD14 55A (GB).
<b>(22) International Filing Date:</b> 6 November 1997 (06.11.97)			
<b>(30) Priority Data:</b>			
08/744,481 6 November 1996 (06.11.96) US			
08/746,036 6 November 1996 (06.11.96) US			
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08/744,590 6 November 1996 (06.11.96) US			
08/786,988 23 January 1997 (23.01.97) US			
08/787,639 23 January 1997 (23.01.97) US			
08/933,792 19 September 1997 (19.09.97) US			
08/947,801 8 October 1997 (08.10.97) US			
<b>(71) Applicant (for all designated States except US):</b> SEQUENOM, INC. [US/US]; 11555 Sorrento Valley Road, San Diego, CA 92121 (US).			<b>(74) Agent:</b> SEIDMAN, Stephanie, L.; Brown Martin Haller & McClain, 1660 Union Street, San Diego, CA 92101-2926 (US).
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<b>(75) Inventors/Applicants (for US only):</b> KOSTER, Hubert [DE/US]; 8636 C Via Mallorca Drive, La Jolla, CA 92037 (US). TANG, Kai [CN/US]; 8521 Summerdale Road #241, San Diego, CA 92126 (US). FU, Dong-Jing [CN/US]; 10615 Dabney Drive #21, San Diego, CA 92126 (US). SIEGERT, Carston, W. [DE/US]; Geielstrasse 42, D-22303 Hamburg (DE). LITTLE, Daniel, P. [US/US]; 393 Glendale Lake Road, Patton, PA 18668 (US). HIGGINS, G., Scott [GB/DE]; Haselweg 1, D-22880 Weidel (DE). BRAUN, Andreas [DE/US]; 13232 Benchley Road, San Diego, CA 92130 (US). DAMHOFFER-DEMAR, Brigitte			
<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).			
<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>			
			<b>(88) Date of publication of the international search report:</b> 22 October 1998 (22.10.98)
<b>(54) Title:</b> DNA DIAGNOSTICS BASED ON MASS SPECTROMETRY			
<b>(57) Abstract</b>  Fast and highly accurate mass spectrometry-based processes for detecting a particular nucleic acid sequence in a biological sample are provided. Depending on the sequence to be detected, the processes can be used, for example, to diagnose a genetic disease or chromosomal abnormality; a predisposition to a disease or condition, infection by a pathogenic organism, or for determining identity or heredity.			

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20444

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C07H21/00 C07F9/24

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

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- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

29 July 1998

Date of mailing of the international search report

28.08.98

Name and mailing address of the ISA

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Authorized officer

Osborne, H

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International Application No

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 29431 A (SEQUENOM INC) 26 September 1996	1
X	pages 25-54	2-7,
	see page 9, line 33 - page 10, line 2;	11-18,
	claims 1-49; figure 9	82,83
X	see page 15, line 34 - page 18, line 10;	19-34,
	examples 5,8	82,83
X	see example 8	42
X	see page 16, line 4; figures 6A,8	47
X	page 36, ln 33	48,49,
	see page 26, line 7	80,81
X	see page 16 - page 18, line 10; figures 3-9	50-64,
		68-70
Y	see page 21, line 21 - line 23	35-37
Y	see example 7	38,39
Y	see claims 1-49	40,41,
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		11-18,
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	especially page 21, lns 12-24.	
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Y	WO 95 13381 A (GERON CORP) 18 May 1995	35-37,
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	see page 9, line 1 - line 33	
	see page 29, line 34 - page 32, line 5	
	see claims 1-4; example 3	
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International Application No

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Y	PASINI B ET AL: "RET mutations in human disease" TRENDS IN GENETICS, vol. 12, no. 4, April 1996, pages 138-144, XP002072975 see page 141, paragraph 2 - page 144 ---	35,38, 39,82,83
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Y	NELSON R ET AL: "Time-of-flight Mass spectrometry of nucleic acids by laser ablation and ionization from a frozen aqueous matrix " RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 4, September 1990, pages 348-351, XP002072976 see abstract ---	42,82,83
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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 15400 A (UNIV JOHNS HOPKINS) 8 June 1995 see abstract and claims 1-19 ---	43-45, 82,83
Y	WO 96 10648 A (PROMEGA CORP) 11 April 1996 see claims 1,23 ---	43-45, 82,83
Y	WO 93 23563 A (CEMU BIOTEKNIK AB ;UHLEN MATHIAS (SE); PETTERSSON BERTIL (SE)) 25 November 1993 see claims 1-7; figure 1 ---	46,82,83
Y	DE 44 38 630 A (PACHMANN KATHARINA DR ;GOEHLY URSULA (DE)) 2 May 1996 see claim 1; figures 1A,1B ---	46,82,83
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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/20444

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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E	WO 97 42348 A (SEQUENOM INC) 13 November 1997 see claims 1-48 -----	1,7-10

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 20444

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

SEE ANNEXES

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

it should be further noted that "claims 82-83" as designated herein refer to two claims 82 and two claims 83 which were filed as follows, claims 82, 83 followed by a second claim 82 and a second claim 83)

1. Claims 1-18, partially 82-83:

A method for determining the sequence of a target nucleic acid involving the generation of base specifically terminated fragments.

2. Claims 19-34, partially 82-83:

A method for detecting a target nucleic acid present in a biological sample based on a nested polymerase chain amplification reaction.

3. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting telomerase), claims 36 and 37, and partially 82-83:

An assay for the detection of neoplasia/malignancies based on telomerase specific extension of a substrate primer and a subsequent amplification of the telomerase specific extension product by PCR.

4. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting mutation of a proto-oncogene), claims 38 and 39, and partially claims 82-83:

An assay for the detection of neoplasia involving mutation analysis of mutant or wild-type alleles by primer extension reaction by a Sanger type sequencing protocol.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting expression of a tumour-specific gene in a specific tissue type), claims 40 and 41, and partially claims 82-83:

An amplification based assay for the expression of the tyrosine hydroxylase gene in bone marrow cells as indicative of a neuroblastoma.

6. Claim 42, partially claims 82-83:

A method for directly detecting double stranded nucleic acid using Maldi-TOF mass spectrometry.

7. Claims 43-46, partially claims 82-83:

A method for comparing DNA relatedness by amplification of microsatellite DNA repeat sequences.

8. Claim 46, partially claims 82-83:

A method for detecting mutations based on target amplification using a primer that introduces a unique endonuclease restriction site into amplified target and a combination of a Sanger sequencing protocol and endonuclease digestion.

9. Claim 47, partially claims 82-83:

A method for the amplification and detection of a nucleic acid based on the synthesis of RNA using a primer containing a RNA polymerase promoter sequence.

10. Claims 48, 49, 80 and 81, partially 82-83:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Primers per se for mass spectrometry comprising a mass modifying moiety.

11. Claims 50-64, partially 68-70, partially 73-79, partially claims 82-83:

Methods for detecting a target nucleic acid sequence involving hybridisation to a detector oligonucleotide.

12. Claims 65-67, partially 68-70, 71-72, partially 73-79, partially claims 82-83:

Methods for determining a nucleic acid sequence involving exonuclease digestion.

13. Claims 84-94:

Photolabile linkers per se for use in immobilisation of nucleic acids to solid supports.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/20444

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